Original Article **Asian J Agri Biol, 2013, 1(4):187-189.**

GLYCATION INHIBITION BY *NIGELLA SATIVA* (LINN)–AN *IN VITRO* MODEL

Hira Zafar[1], Fatma Hussain[1*], Shoaib Zafar[2], Riffat Yasmin[1]

[1]*Department of Chemistry and Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan*

[2]*Department of Physiology and Pharmacology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan*

ABSTRACT

In present study, an *in vitro* glycation model comprising both plasma and glucose designed to assess glycation inhibitory potentials of *N. sativa* seed (NS) extracts. Plasma with several glucose quantities in the presence of NS were incubated for thirty-five days at 37°C. Non-enzymatic glycation (NEG) was quantified by Thiobarbituric acid technique. NEG levels were reduced by NS. Higher NS concentrations significantly ($P < 0.05$) reduced the NEG demonstrating dose-dependent inhibitory potential of NS. Plasma NEG levels were decreased up to 13% ($P< 0.05$). NEG inhibition correlated ($r=0.784$) with NS concentrations. Present glycation inhibitory prospective of NS demands further studies to determine the precise mechanism of action and effective dose of NS for therapeutic interventions.

Keywords: Glycation inhibition, Hyperglycemia, Human plasma, *Nigella sativa*

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders affecting millions worldwide (Uma and Sudarsanam, 2012). Hyperglycemia has a key role in non-enzymatic glycation (NEG) of lipids, proteins and nucleic acids. Diabetic complications are greatly enhanced with increase NEG and oxidative stress (Wu *et al.,* 2011). In this regard, several natural compounds known to possess antioxidative and antiglycation properties, such as curcumin, rutin, garcinol and flavonoid-rich extracts, have been shown to prevent glycation formation *in vitro* and *in vivo*. These agents that inhibit the formation of protein glycation are purportedto have therapeutic potentials in patients with diabetes and related diseases (Kim and Kim, 2003; Akash *et al.,* 2011; Mathur *et al.,* 2011).

Nigella sativa (family Ranunculaceae) is generally known as "black cumin." *Nigella sativa* seed (NS) is used as a flavoring spice and for the management of diabetes mellitus, infection and cancer. *N. sativa* may be beneficial in diabetic individuals and those with glucose intolerance as it reduces appetite, intestinal glucose absorption, hepatic gluconeogenesis, blood glucose level, cholesterol, triglycerides, body weight and simulates glucose induced secretion. In streptozotocin (STZ) induced diabetic rats it caused partial regeneration of pancreatic beta-cells, increased the lowered serum insulin concentrations and decreased the elevated serum glucose. *N. sativa* has antioxidant activity and protective role against development of diabetes mellitus (Mathur *et al.,* 2011; Javed *et al.,* 2012). However, limited data is available on antiglycation activity of NS. Therefore, the present study was conducted to assess *in vitro* protein glycation inhibitory activity of *Nigella sativa*.

MATERIALS AND METHODS

Extract preparation

NS were washed with distilled water, air-dried and ground into powder. NS powder mixed with distilled water was kept on orbital shaker for 48 hours at 120 rpm and room temperature. Mixture was filtered and filtrate was evaporated to dryness. Before analysis, it was reconstructed as I_1 (500 mg/mL), I_2 (250 mg/mL) andI_3 (50 mg/mL) (Mensor *et al.,* 2001).

Collection of Samples

Healthy normoglycemic volunteers provided blood samples after consent. Plasma was separated and all the plasma samples were pooled together.

Incubations

Plasma samples were incubated with glucose (5.5, 25 and 50m*M*) and NS extracts (50, 250, 500 mg/ml) in phosphate buffer saline (PBS)

**Corresponding author:* e-mail: fatmauaf@yahoo.com

at37°C for five weeks (Table 1).Glucose was used as glycation agent. While, NS acted as an inhibitor. At the end of incubation period, glucose concentrations were measured. Another glucose measurement was done after dialysis to validate final glucose levels. Total protein bioassay was conducted by Biuret method (Gornall *et al.,* 1949).

Glycation analysis

Glycation was determined by TBA (Thiobarbituric acid colorimetry) test (Furth, 1988).

***1. Glycation* (*NEG + EG*)**

One ml sample (after dialysis and protein levels as 10 mg/ml) was used. Three test tubes were arranged for reduced and three for non-reduced samples. $NaBH_4$ and 0.01N NaOH (0.1ml each) was used for reduced and non-reduced samples respectively. After incubation (37°C, 30 minutes), 10 µL of 1N HCl, 0.5 ml oxalic acid were addedand autoclaved at 124°C (ll5 Lb /inch^2 pressure, 30 minutes). After cooling, 40% trichloroacetic acid (0.5mL) was added and centrifuged (5000 rpm) for 15 minutes. Supernatant (1500µL) was mixed with same amount of TBA. After incubation at 37°C (15 minutes), absorbance was taken at 443 nm (Furth, 1988).

***2. Enzymatic Glycation* (*EG*)**

For EG, 0.1 ml NaOH (0.01N) with excess of $NaBH_4$ was used. The glycation level was determined by TBA test (Furth, 1988).

***3. Non-enzymatic glycation* (*NEG*)**

Non-enzymatic glycation was determined (Furth, 1988) as follows:

NEG=(NEG+EG)–EG

All data were expressed as mean ±SE of triplicate measurements. Statistical analyses was performed by SPSS software (version 15.0) with level of significance set at p <0.05.

RESULTS

Results of the present study are presented in figure 1. BSA was used as negative control. Normal plasma (P_N+G_1, PN+G_2, PN+G_3 as positive control) incubated with 50, 25 and 5.5m*M* glucose had 4.07 ± 0.068, 3.65 ± 0.017 and 3.09 ± 0.05 (mole glucose/mole protein) NEG levels. By using I_1, NEG was significantly (P<0.05, 7.11-13%) decreased as compared to positive controls. Decline in NEG remained significant (P<0.05, 3.23-9.33%) when diluted inhibitor concentration (I_2) was used. Although, I_3 (50 mg/ml) was 10 times diluted as compare to the I_1, it had effective (P<0.05, 8.1%) inhibitory activity. Results indicated dose-dependent inhibitory potential of NS, as I_1halted NEG more efficiently as compared to I_2 and I_3. Similarly, G^1generatedmaximum NEG as compared to other glucose concentrations (G_2, G_3). NEG inhibition correlated (r=0.784) with NS concentrations.

DISCUSSION

The non-enzymatic glycation or maillard reaction is associated with diabetes related complications. The investigation of natural glycation inhibitors may provide new avenues for drug discovery (Wu *et al.,* 2011).

The effect of NS on glycation induced damage is never studied. In the present study, plant extracts demonstrated antiglycation activity when tested by plasma-glucose assay. The mechanism of NS antiglycation activity was not explored in the present study. Glycation inhibitory activity of NS may be due to different phenolic components. Numerous studies have shown that many herbs and spices have high polyphenol content and antiglycation activities. Glycation inhibition by plants can be due to their antioxidant activity or by a carbonyl blocking mechanism which prevents the formation of amadori products (Kim and Kim, 2003; Lunceford and Gugliucci, 2005; Thomas *et al.,* 2005).

Wu *et al.,* 2011 investigated the inhibitory effect of naturally occurring flavonoids on protein glycation using *in vitro* models. The results indicated that protein glycation inhibitory capability of flavonoids was remarkably related to the scavenging free radicals derived from glycoxidation process.

Our data is partially in accordance with the Losso *et al.,* (2011). They demonstrated that thymoquinone,a major quinone from *Nigella sativa* has an inhibitory action against the glycation process. At 20 µM, thymoquinone inhibited 39% of hemoglobin glycation.

Earlier, *in vitro* glycation was studied by Vinson and Howard (1996) with BSA (bovine serum albumin) with glucose or fructose as glycating agents. Eighteen normal subjects were supplemented with 1000 mg of ascorbic

acid for a period of 4 weeks. Serum protein glycation was decreased an average of 46.8% ($p < 0.01$). These results underline the importance of nutrition in diabetes and indicate the possibility of therapeutic use of these nutrients for the prevention of diabetic complications.

Present study demonstrates the possibility of inhibiting NEG with NS seeds extracts *in vitro.* Furthermore, long-term in vivo studies are needed to demonstrate the usefulness of NS supplementation for the prevention of diabetic complications.

CONCLUSION

NS was demonstrated to possess antiglycation activity based on an *in vitro* glycation model. However, the mechanism of NS antiglycation activity and its application as a therapeutic agent need further investigation. It is hoped that such therapies will be fully evaluated in the clinical context with the ultimate aim to reduce the major economical and medical burden of diabetes mellitus.

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